
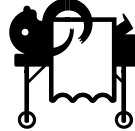










# The Importance of Molecular Typing

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Legionellosis Webinar #3  
November 17, 2010

# Molecular typing during epidemiological investigation

	State A	State B	State C
Clinical investigations and cases reported to CDC	Patient 1 	Patient 2 	
Environmental investigations			Putative sources Hotel X  Hotel Z  Cooling tower 
Species identification	 <i>L. pneumophila</i>	 <i>L. pneumophila</i>	 <i>L. pneumophila</i>  <i>L. pneumophila</i>  <i>L. pneumophila</i>

Modified from Norman Fry *et al*, 1999, *Clin. Microbiol. Infect.* 5: 462-477

# When should molecular typing be pursued?

- to confirm that isolates from cases are identical (cases are exposed to the same source)
- to compare clinical to environmental isolates to narrow down the list of potential environmental sources

# Molecular methods for typing *Legionella* isolates

- Monoclonal antibody (MAb) typing
- DNA fragment-based methods:
  - PFGE pulse-field gel electrophoresis
  - RFLP restriction fragment length polymorphism
  - AFLP amplified fragment length polymorphism
  - MLVA multi-locus variable-number tandem repeat analysis
- DNA sequencing methods:
  - *mip* gene sequencing
  - SBT sequence based typing (*L. pneumophila*)
- Peptide fragment-based method

# Criteria to consider when choosing typing methods:

- How easy to perform
- How long does it take
- Cost
- Discriminatory power
- Interlaboratory comparison (is it portable)

# Monoclonal antibody typing

International panel of seven MAbs established in 1986

Test for *L. pneumophila* reactivity with seven MAbs



(1,4,7) Bellingham


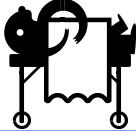








# Monoclonal antibody typing

## Advantages

- Easy
- Fast (15-20 min)
- Cheap
- Portable if different labs use the same MAbs

## Disadvantages

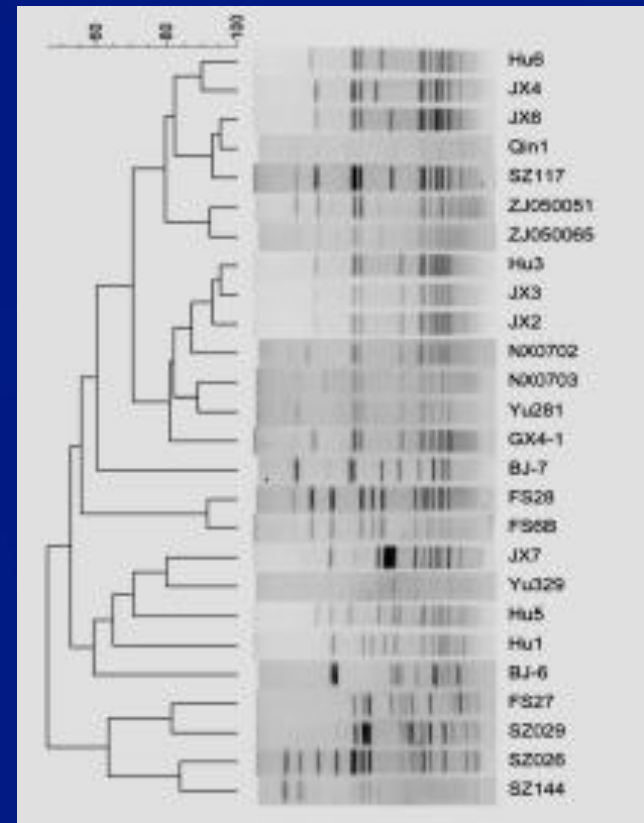
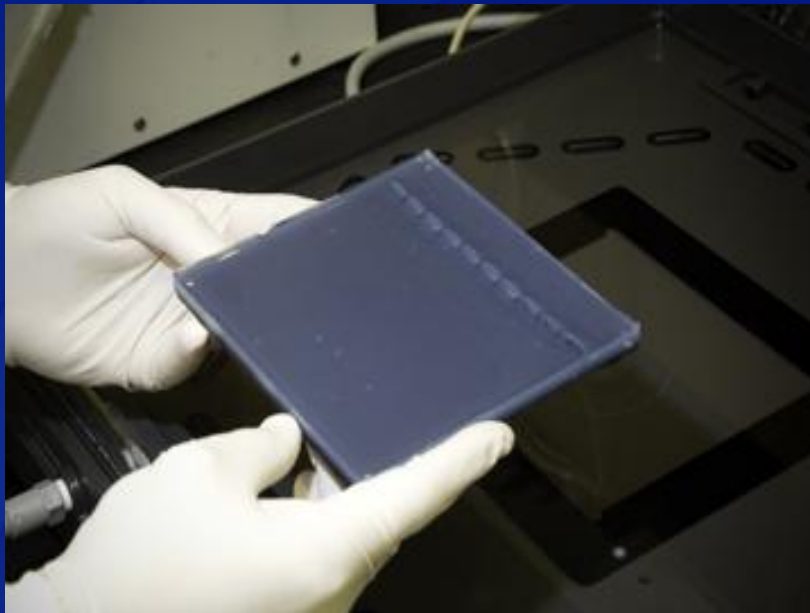
- MAbs are not commercially available
- Only for *L. pneumophila* serogroup 1
- Only 12 MAb patterns

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MAb typing of <i>L. pneumophila</i> sg1 isolates	(1,2,5) sg1, Allentown	(1,2,5) sg1, Allentown	(1,2,5) sg1, Allentown	(1,4,7) sg1, Bellingham	(1,2,5) sg1, Allentown

# PFGE

Genomic DNA is cut with restriction enzymes

DNA fragments are separated by gel electrophoresis with the voltage periodically switching directions



# PFGE

## Advantages

- Cheap
- High discriminatory power
- Could be used for all *Legionella* species

## Disadvantages

- Time-consuming
- Could be difficult to interpret banding pattern
- Not portable

# Sequence Based Typing (SBT)

PCR-amplification and sequencing of seven *L. pneumophila* gene fragments: *flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA*

Based on the sequence, each gene fragment is assigned an allele number, which results in an **allelic profile** (e.g. 1,4,3,1,1,1,1) and a corresponding **sequence type (ST)**, e.g. ST1

Supported by the European Working Group for *Legionella* Infection (EWGLI)



# Sequence Based Typing (SBT)

[http://www.hpa-bioinformatics.org.uk/legionella/legionella\\_sbt/php/sbt\\_homepage.php](http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php)

## *Legionella pneumophila* Sequence-Based Typing

Welcome to the EWGLI **Sequence-Based Typing (SBT) Database** for *Legionella pneumophila*

A consensus Sequence-Based Typing (SBT) epidemiological typing scheme for clinical and environmental isolates of *Legionella pneumophila* has been developed by members of the European Working Group for Legionella Infections (EWGLI) and evaluated for implementation in the investigation of outbreaks of legionellosis caused by *L. pneumophila*.

Using the SBT protocol, the SBT database (version 3.0) allows assignment of the seven ordered alleles, *flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA* as described by Gaia *et al.* (2005) and Ratzow *et al.* (2007), represented as a Sequence Type (ST), or allelic profile, of the ordered string of allele numbers separated by commas e.g. 1,4,3,1,1,1,1.

The curators encourage the submission of putative new alleles. Submission of putative new alleles can be made via the [Sequence Quality Tool](#) or by the New Allele Submission link (Options menu, left), which examines the forward and reverse chromatogram files. Subject to verification by the curators, a new allele number will be assigned and added to the database. If the curators are unable to verify a new allele, the strain or genomic DNA may be requested to allow sequencing by another designated centre. Submission of strains bearing new allele numbers to the EUL culture collection is strongly encouraged.

Please contact [Dr. Norman Fry](#) for further details.

Total number of entries:	4334	Sample source, total number of records 4334	
Number of <b>Sequence Types</b> :	852		
Number of <i>flaA</i> alleles:	28		
Number of <i>pilE</i> alleles:	41		
Number of <i>asd</i> alleles:	45		
Number of <i>mip</i> alleles:	51		
Number of <i>mompS</i> alleles:	60		
Number of <i>proA</i> alleles:	37		
Number of <i>neuA</i> alleles:	36		



Provided by The European Working Group for Legionella Infections (EWGLI) in conjunction with



The Health Protection Agency and



The European Centre for Disease Prevention and Control

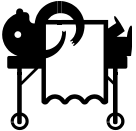
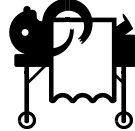








# SBT

## Advantages

- Relatively easy
- High discriminatory power
- Provides reproducible results
- Portable

## Disadvantages

- Sequencing step is expensive: either in-house using genetic analyzer (~ \$100K) or send to DNA sequencing company and pay per reaction
- Only for *L. pneumophila*

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MAB typing of <i>L. pneumophila</i> sg1 isolates	(1,2,5) sg1, Allentown	(1,2,5) sg1, Allentown	(1,2,5) sg1, Allentown	(1,4,7) sg1, Bellingham	(1,2,5) sg1, Allentown
Molecular typing	(3,4,1,1,1,9,1) ST35	(3,4,1,1,1,9,1) ST35	(1,4,3,1,1,1,9) ST8	(3,4,1,1,1,9,1) ST35	

# Take home message:

- It is necessary to use molecular typing to subtype *Legionella* isolates in order to *i*) confirm that isolates from cases are identical and *ii*) compare clinical to environmental isolates
- MAb, PFGE, and SBT are independent and complementary typing methods
- CDC *Legionella* lab combines MAb (fast initial) and SBT (longer final) methods for *L. pneumophila* typing
- The majority of molecular typing methods requires *Legionella* **isolates**

# References:

- Joly, J.R. et al. 1986. Development of a standardized subgrouping scheme for *Legionella pneumophila* serogroup 1 using monoclonal antibodies. *J. Clin. Microbiol.* **23**: 768-771
- Fry, N.K. et al. 1999. A multicenter evaluation of genotypic methods for the epidemiologic typing of *Legionella pneumophila* serogroup 1: results of a pan-european study. *Clin. Microbiol. Infect.* **5**: 462-477
- *Legionella pneumophila* Sequence-Based typing homepage:  
[http://www.hpa-bioinformatics.org.uk/legionella/legionella\\_sbt/php/sbt\\_homepage.php](http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php)

**For more information please contact Centers for Disease Control and Prevention**

1600 Clifton Road NE, Atlanta, GA 30333

Telephone, 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348

E-mail: [cdcinfo@cdc.gov](mailto:cdcinfo@cdc.gov) Web: [www.cdc.gov](http://www.cdc.gov)

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.